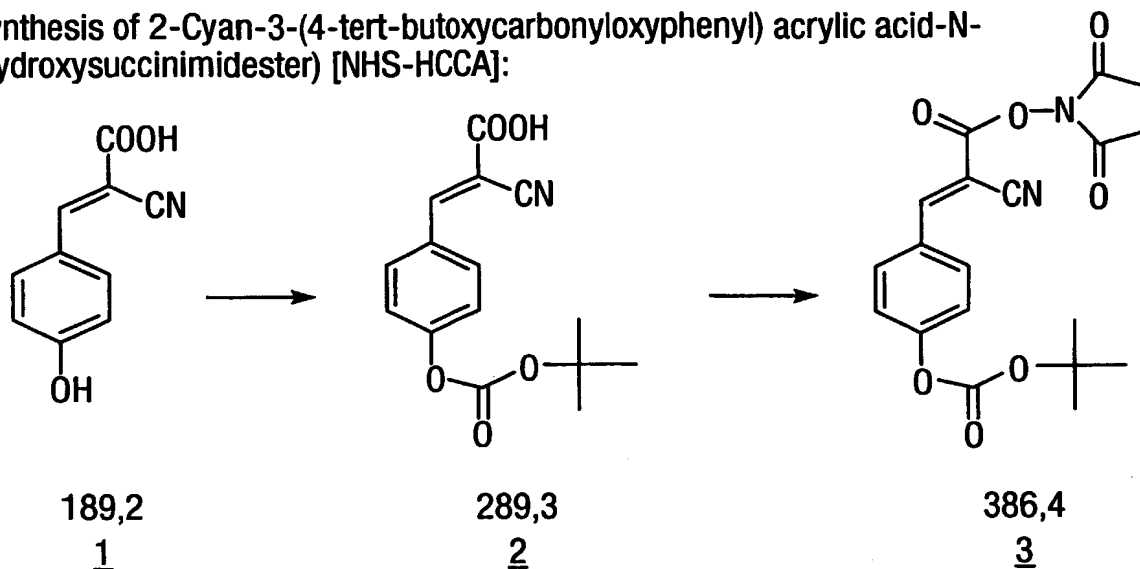


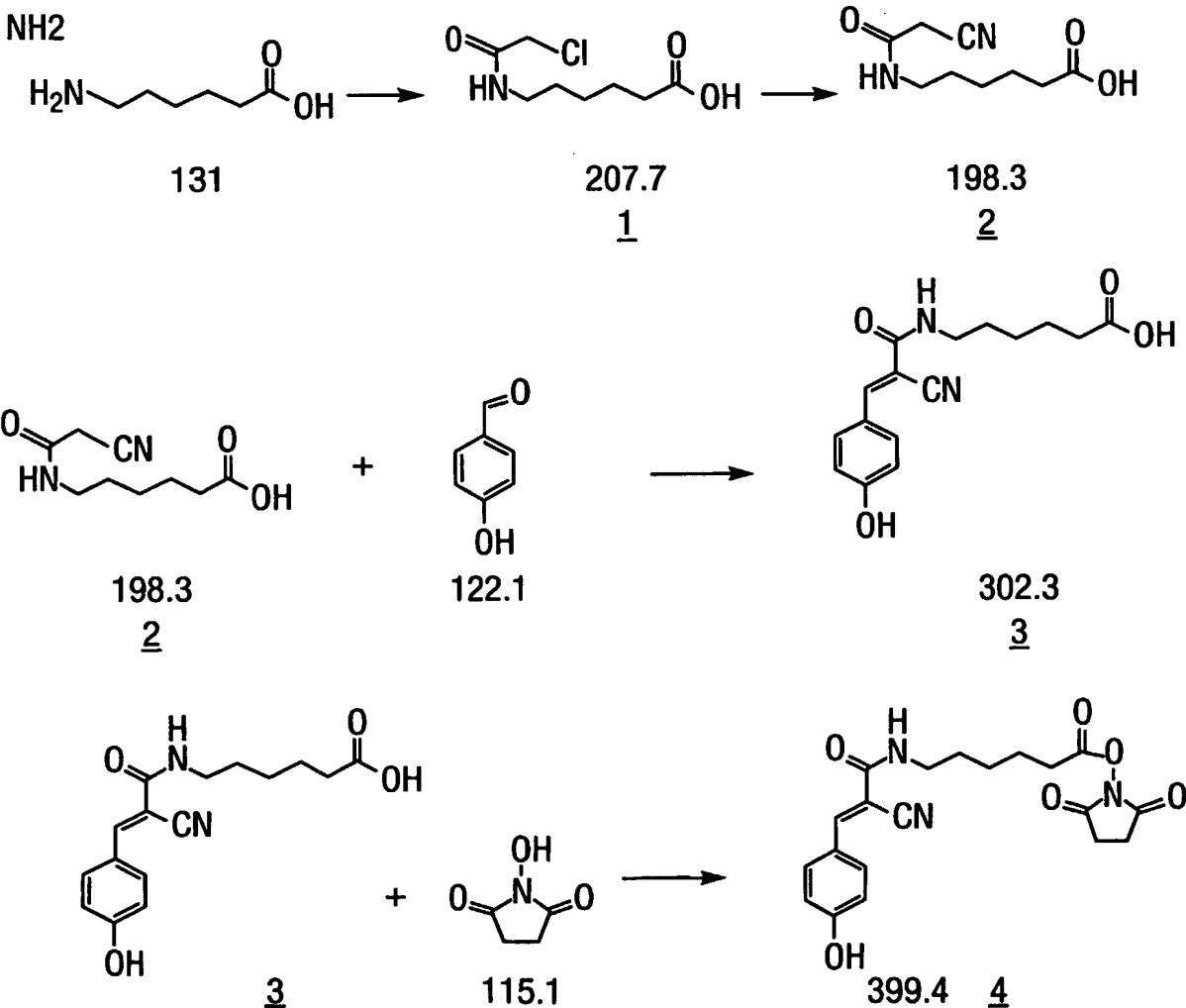
1/22

## Fig.1.

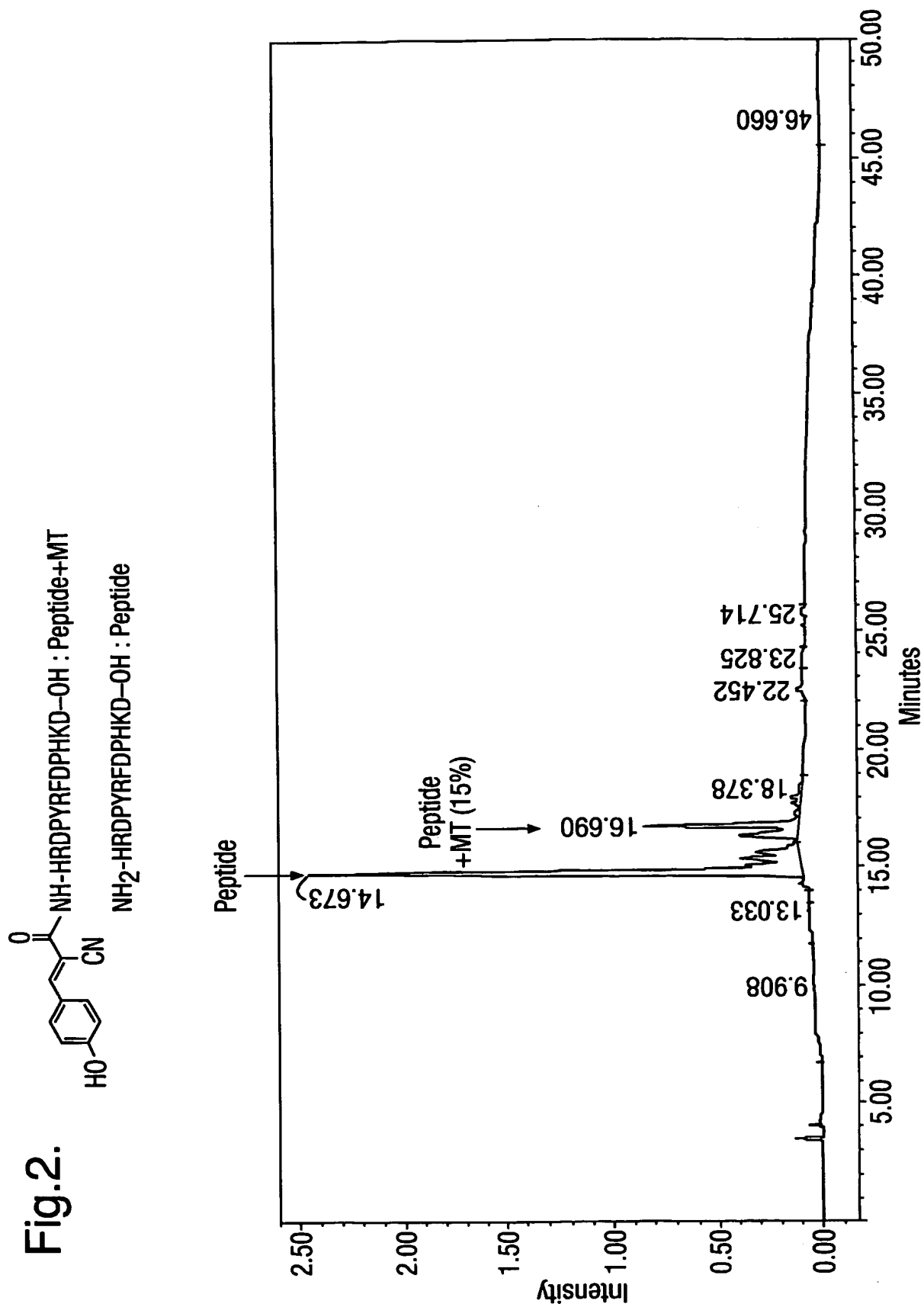
Synthesis of 2-Cyan-3-(4-tert-butoxycarbonyloxyphenyl) acrylic acid-N-(hydroxysuccinimidester) [NHS-HCCA]:



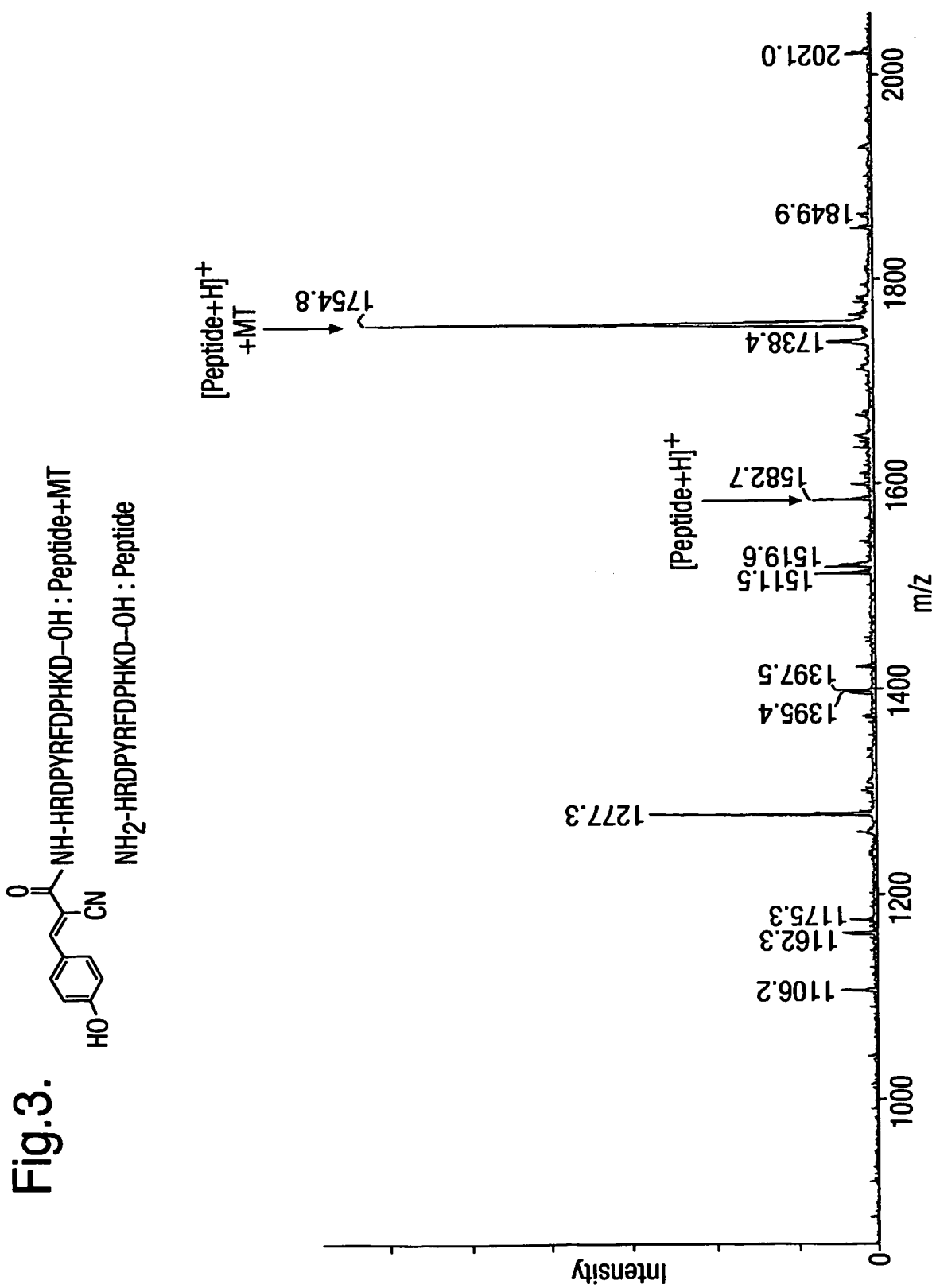
Synthesis of 2-Cyan-3-(4-tert-hydroxyphenyl) acrylic acid-6-[(2,5-dioxo-1-pyrrolidinyl) oxy]-(6-oxo) hexyl] amide [NHS-L-HCCA]:



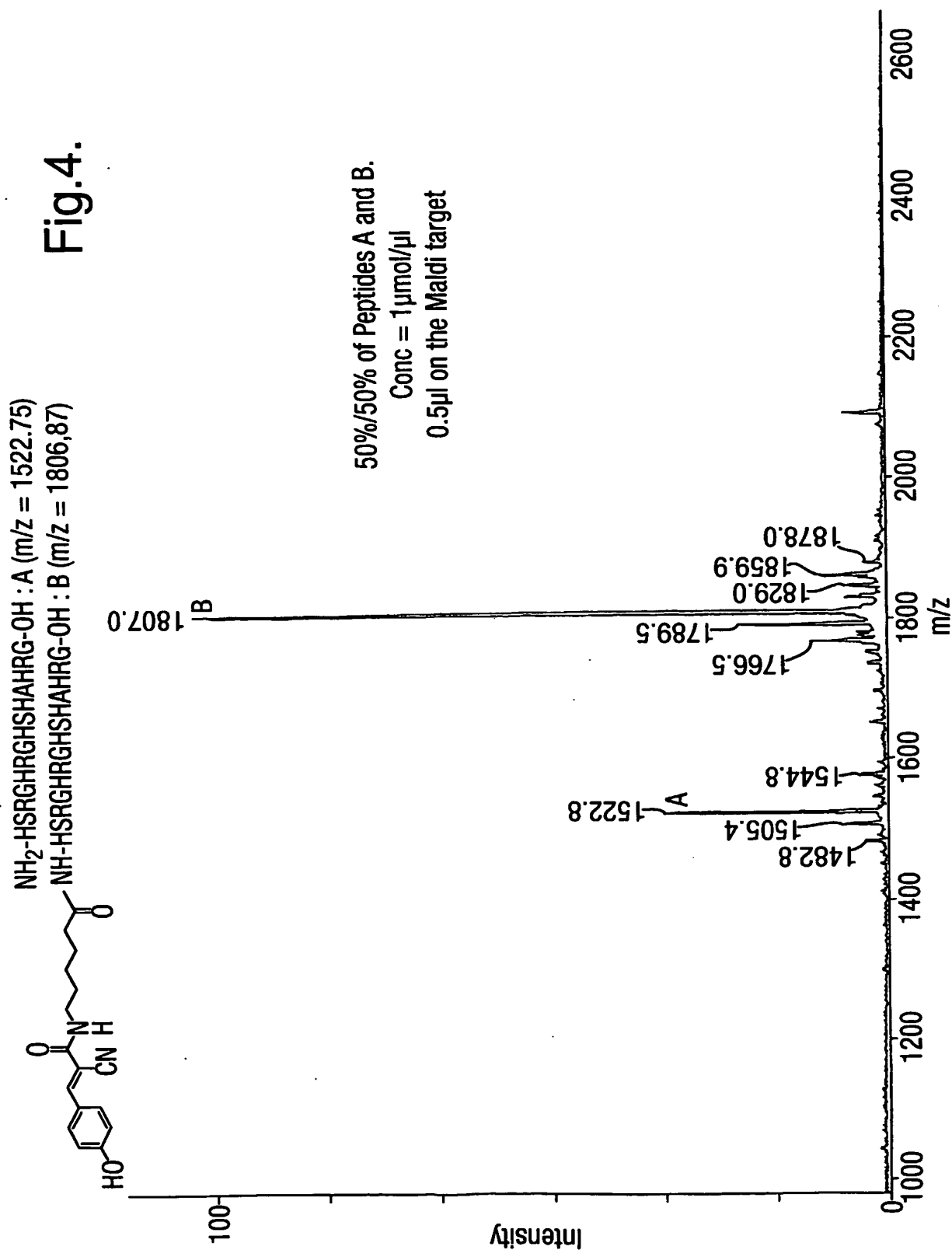
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Fig.5a.

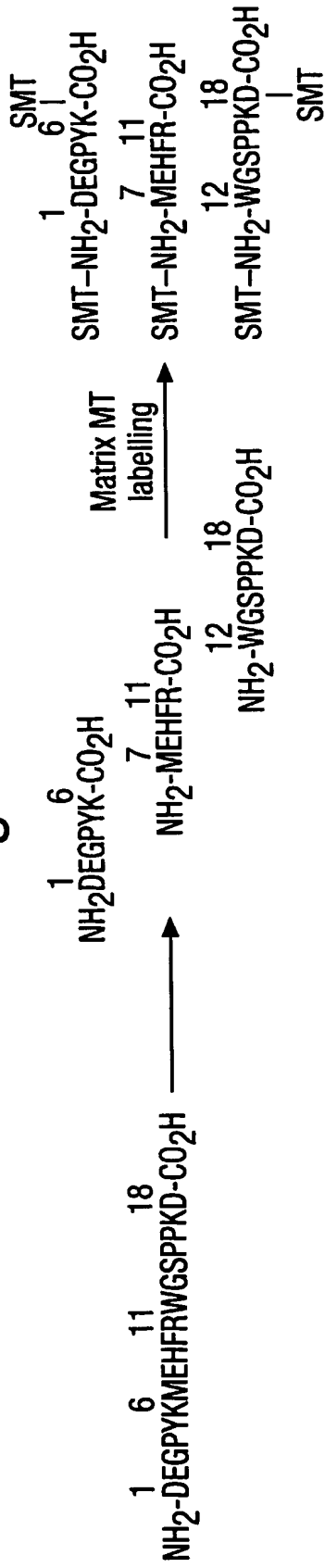
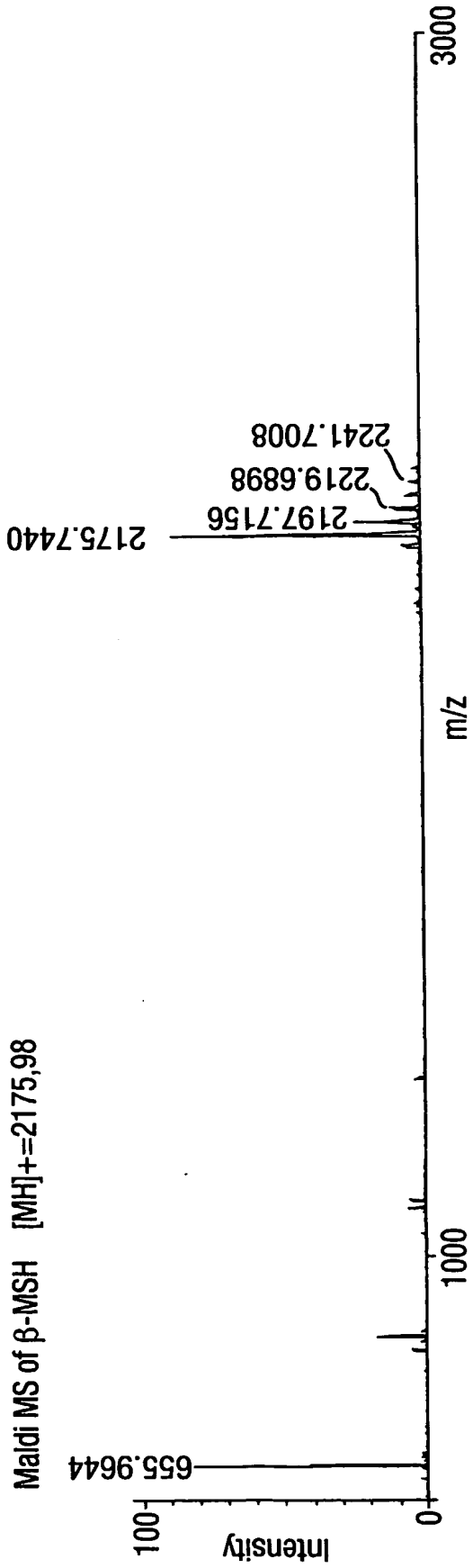


Fig.5b.



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Fig.6.

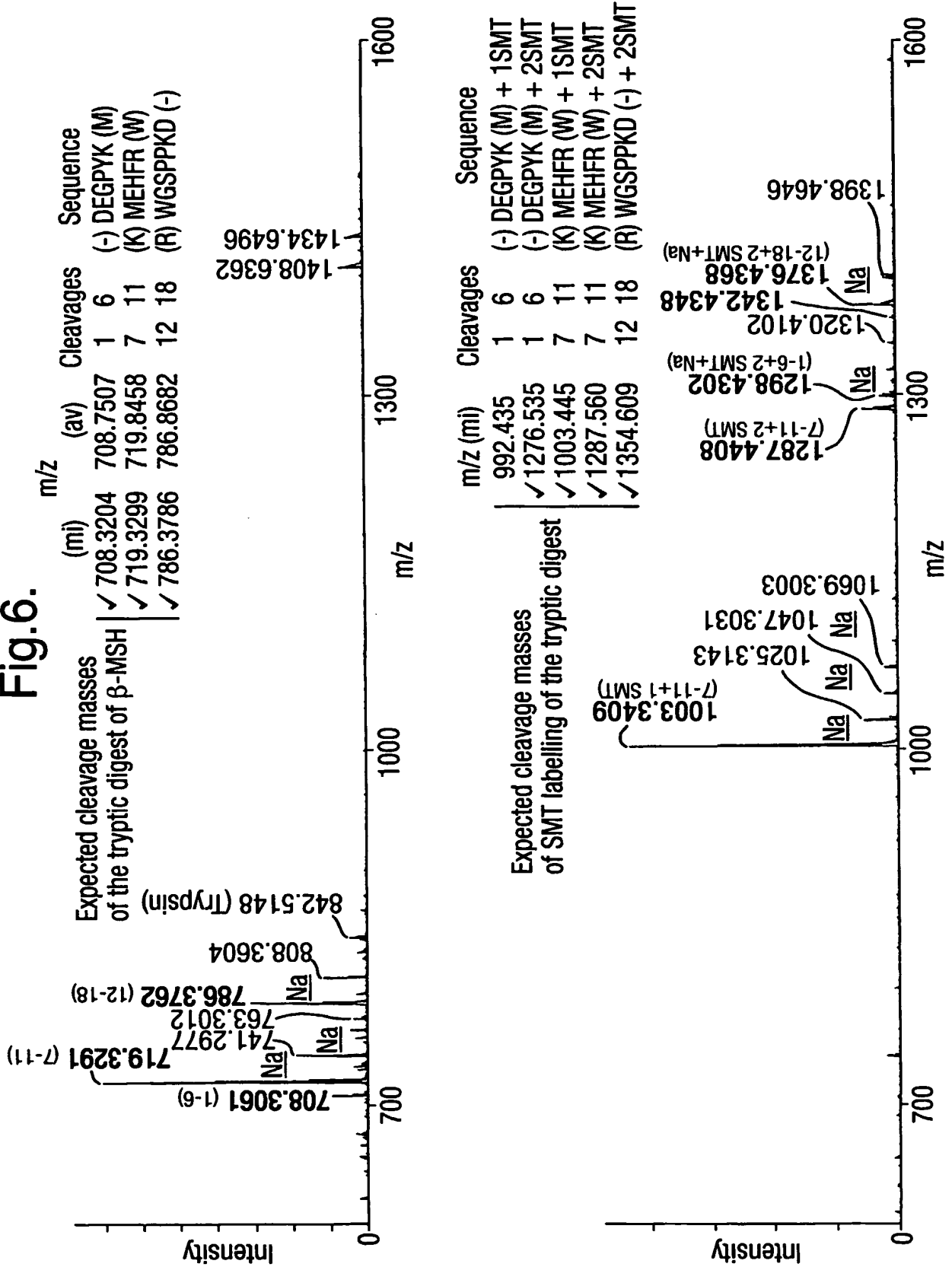
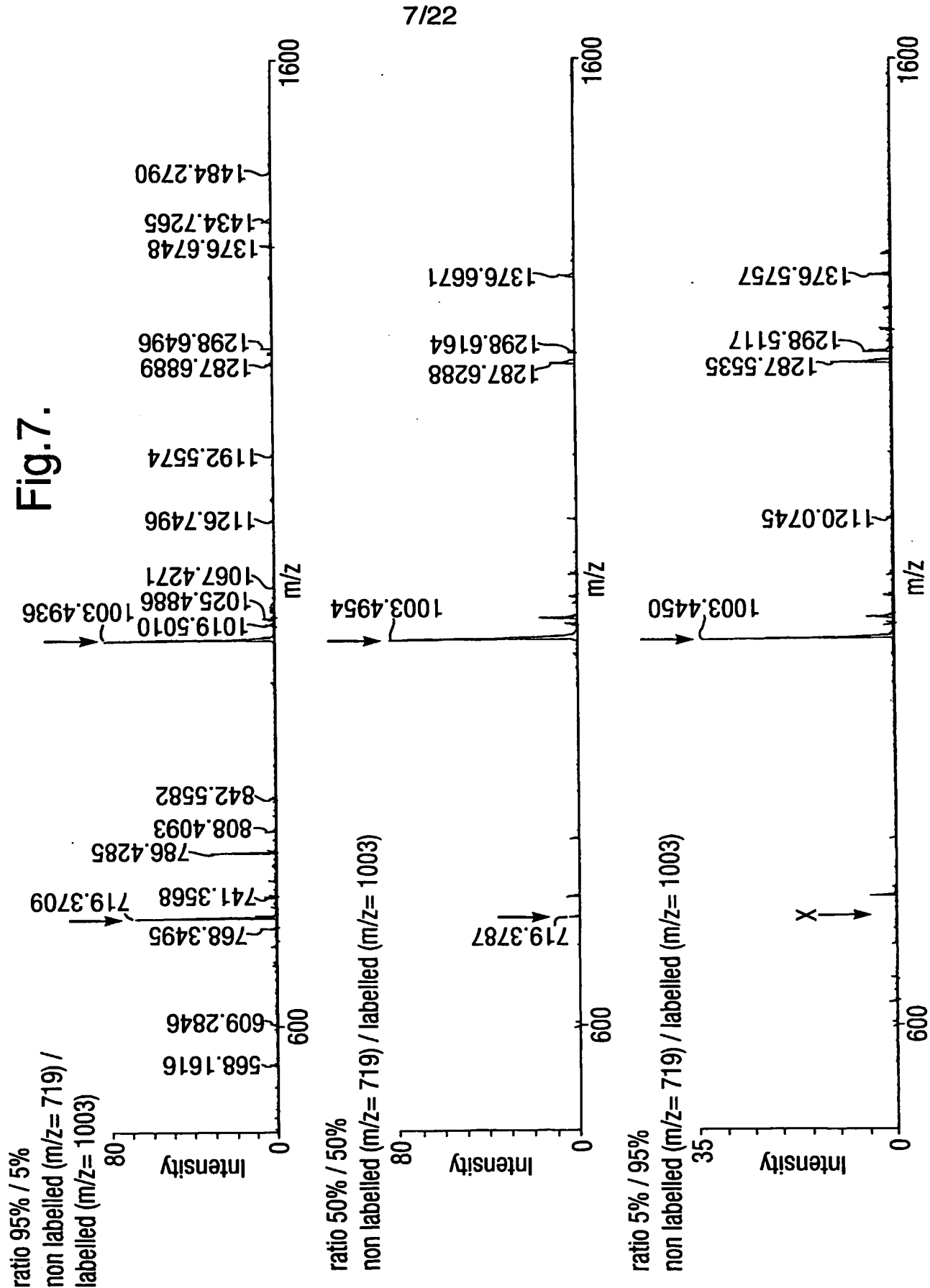


Fig.7.



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Fig.8.

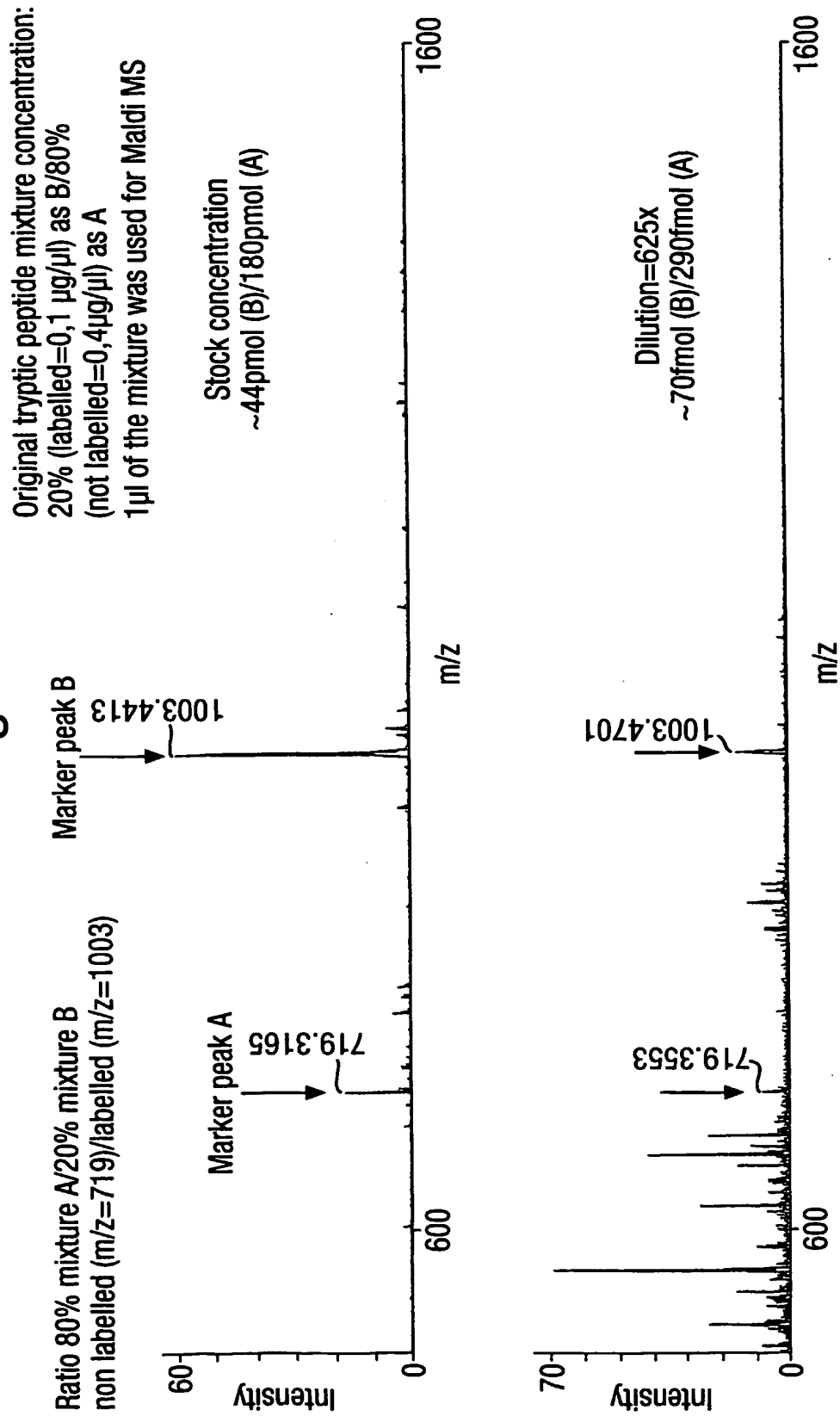
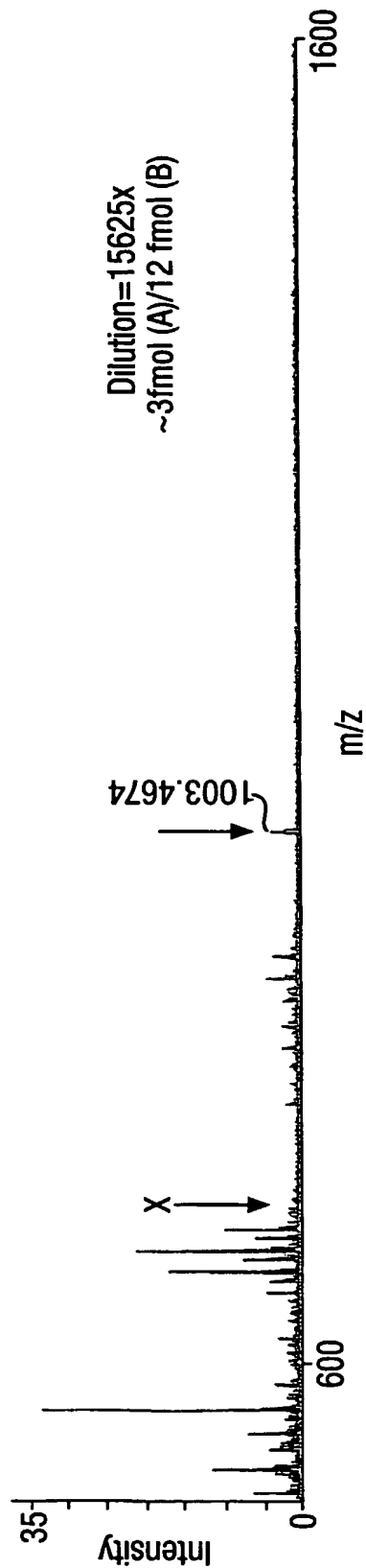
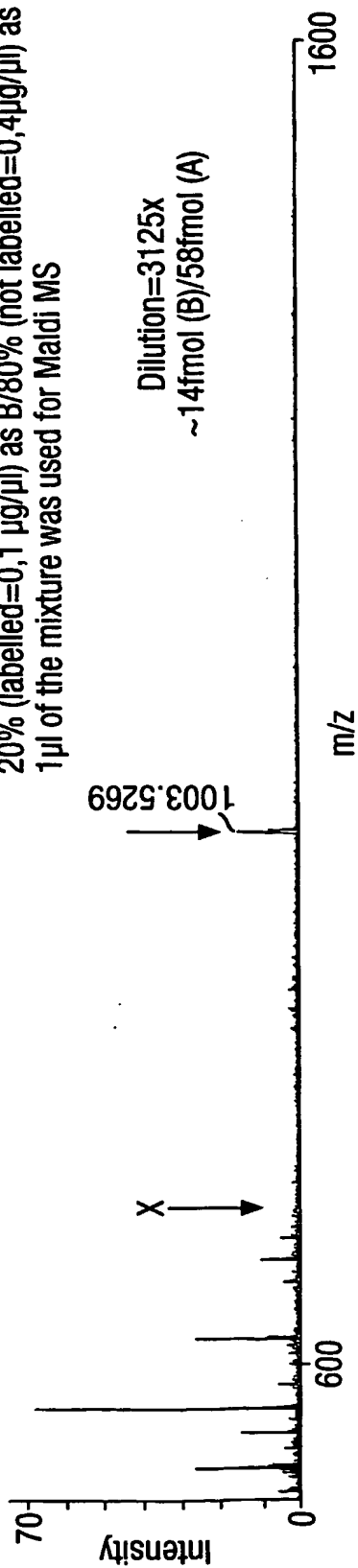




Fig.8 (Cont).

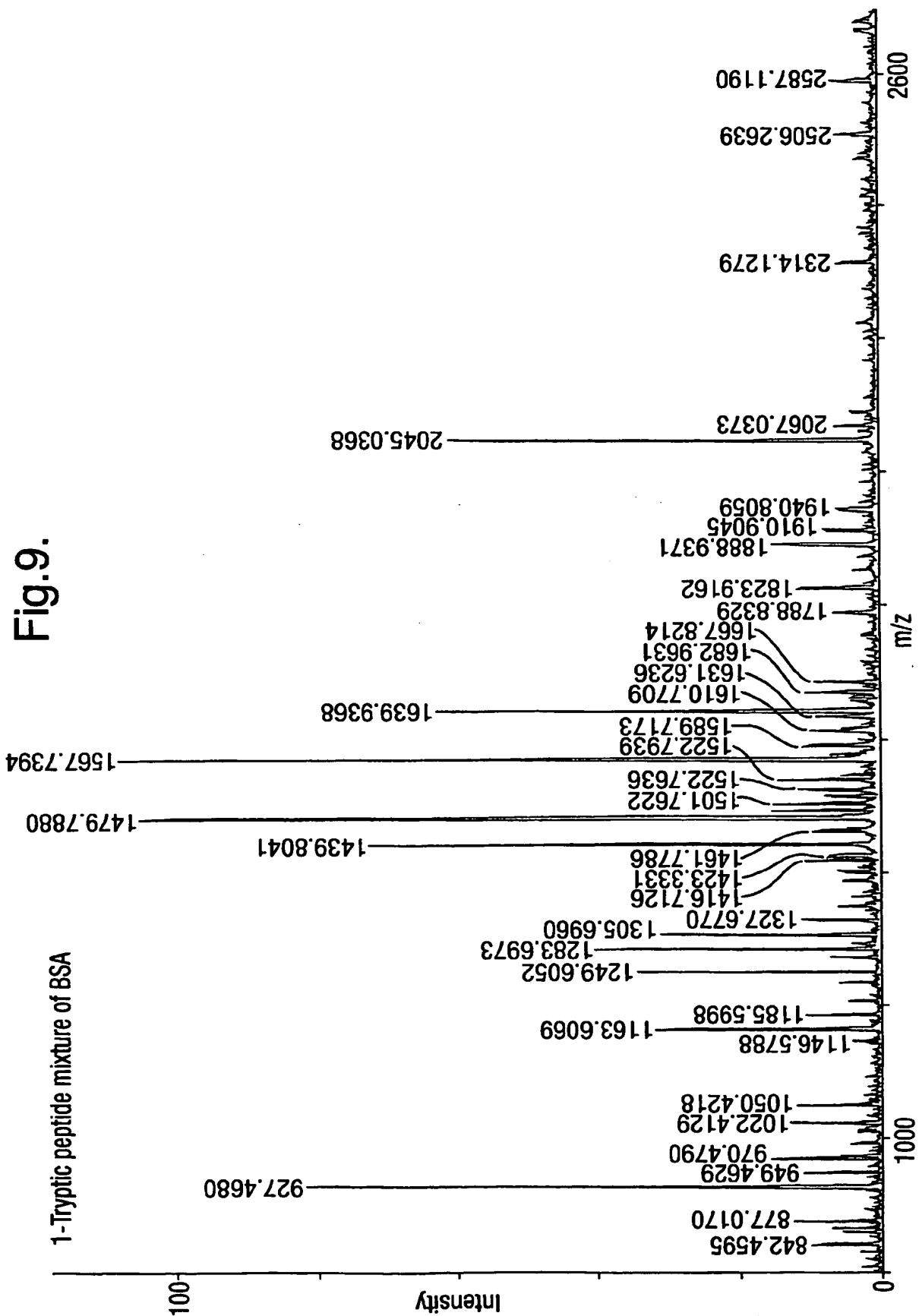
Ratio 80% mixture A/20% mixture B  
non labelled ( $m/z=719$ )/labelled ( $m/z=1003$ )

Original tryptic peptide mixture concentration:  
20% (labelled= $0.1 \mu\text{g}/\mu\text{l}$ ) as B/80% (not labelled= $0.4 \mu\text{g}/\mu\text{l}$ ) as A  
 $1 \mu\text{l}$  of the mixture was used for MALDI MS



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Fig.10.

15/42 matches (35%). 69293.9 Da, pI=5.82. Acc. # swiss:P02769. BOVIN. SERUM ALBUMIN PRECURSOR..

m/z submitted	MH <sup>+</sup> matched	$\Delta$ ppm	start	end	Peptide Sequence	Modifications
927.4680	927.4940	-28.0249	161	167	(K)YL <del>Y</del> EIAR (R)	
1163.6069	1163.6312	-20.8956	66	75	(K)LVNELTEFAK (T)	
1249.6052	1249.6217	-13.2142	35	44	(R)FKDLGEEHFK (G)	
1283.6973	1283.7112	-10.8252	361	371	(R)HPEYAVSVLLR (L)	
1305.6960	1305.7167	-15.8348	402	412	(K)HLVDEPQNLIK (Q)	
1439.8041	1439.8123	-5.7004	360	371	(R)RHPEYAVSVLLR (L)	
1479.7880	1479.7960	-5.3985	421	433	(K)LG <del>E</del> YGFQNALIVR (Y)	
1567.7394	1567.7433	-2.4778	347	359	(K)DAFLGSFLYEYSR (R)	
1639.9368	1639.9383	-0.9122	437	451	(R)KVPQVSTPTLVEYSR (S)	
1667.8214	1667.8137	4.6234	469	482	(R)MPCTEDYLSLILNR (L)	
1823.9162	1823.9002	8.7746	508	523	(R)RPCFSALTPDETYVPK (A)	
1888.9371	1888.9274	5.1368	169	183	(R)HPYFYAPELLYYANK (Y)	
2045.0368	2045.0285	4.0547	168	183	(R)RH <del>P</del> YFYAPELLYYANK (Y)	
2506.2639	2506.2508	5.2276	469	489	(R)MPCTEDYLSLILNRLCVLHEK (T)	
3395.6670	3395.6438	6.8340	558	587	(K)HKPKATEEQ <del>L</del> KTVMENFVAFVDKCCAADDK (E)	1Met-ox

The matched peptides cover 28% (174/607 AA's) of the protein

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Fig.11.

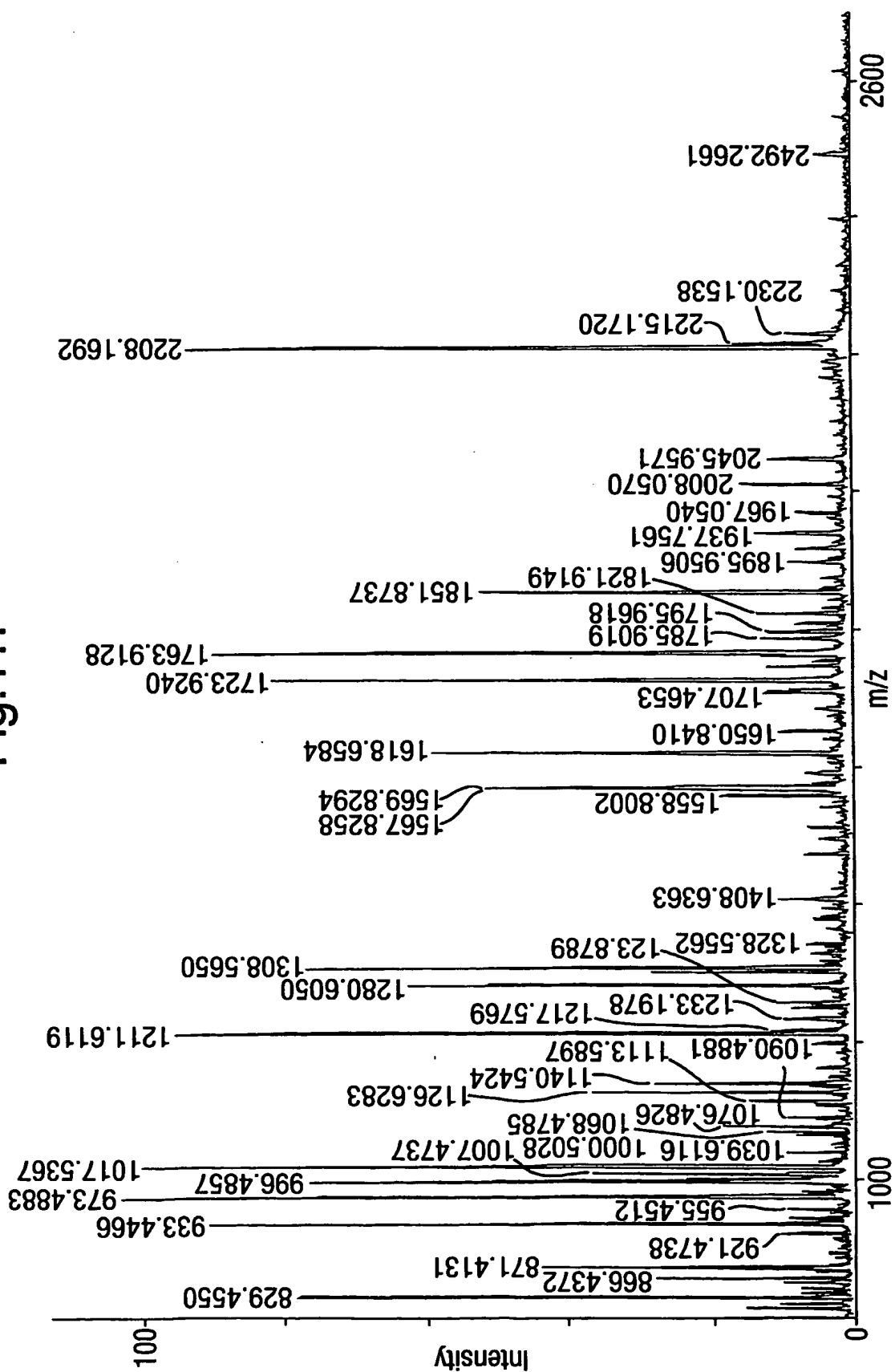


Fig.12.

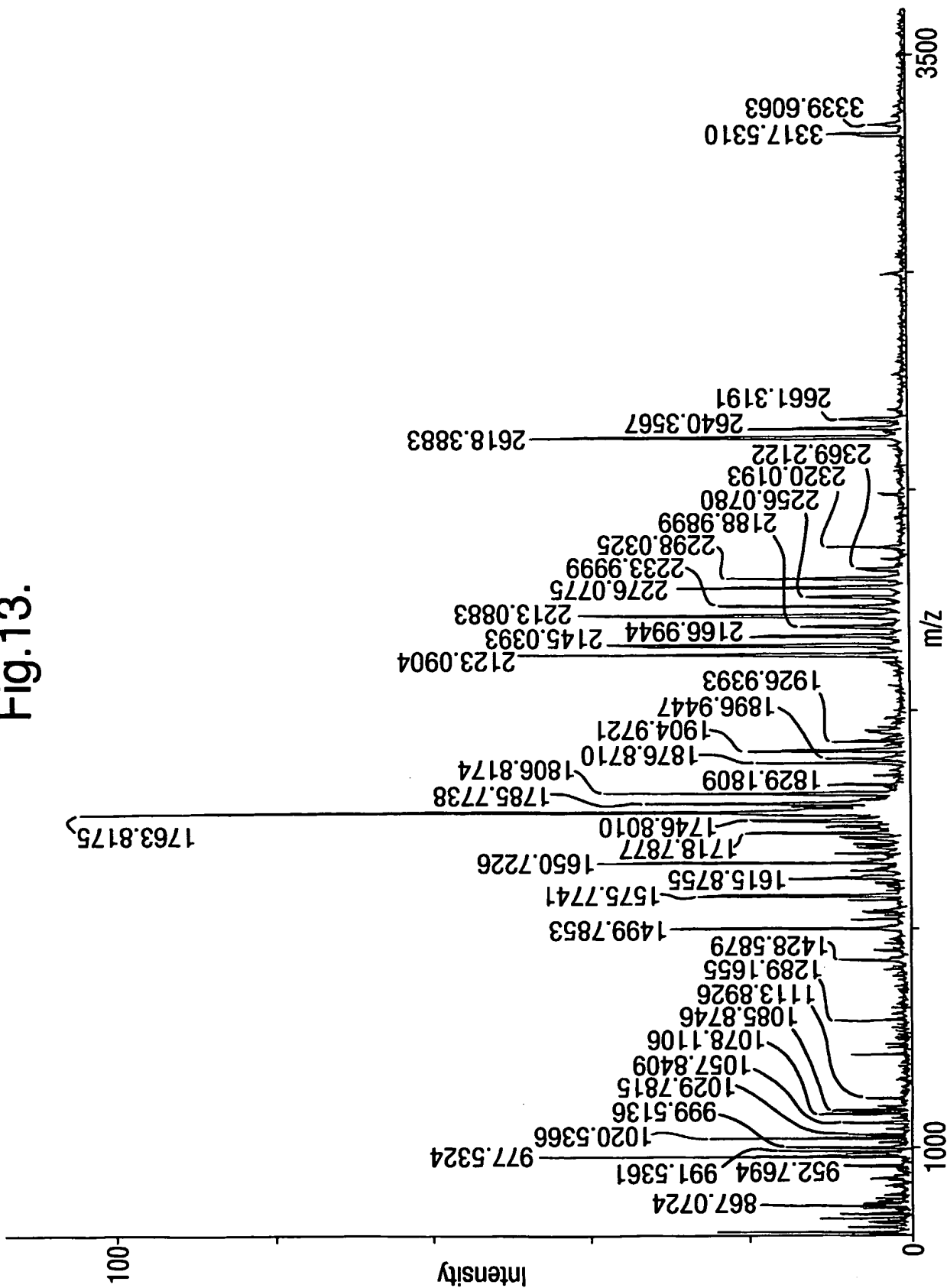
16/50 matches (32%). 69293.9 Da, pl = 5.82. Acc. # swiss: P02769. BOVIN. SERUM ALBUMIN PRECURSOR.

m/z	MH <sup>+</sup>	Δ ppm	start	end	Peptide Sequence	Modifications
829.4550	829.4572	-2.6686	101	105	(K) <u>VASLR</u> (E)	1SMT
933.4466	933.4504	-4.0854	205	209	(K) <u>IETMR</u> (E)	1SMT
933.4466	933.4504	-4.0854	223	228	(R) <u>CASIQK</u> (F)	1SMT
973.4883	973.4896	-1.3110	236	241	(K) <u>AWSVAR</u> (L)	1SMT
996.4857	996.4903	-4.6207	29	34	(K) <u>SEIAHR</u> (F)	1SMT
1068.4785	1068.4791	-0.5345	25	28	(R) <u>DTHK</u> (S)	1SMT
1211.6119	1211.6101	1.4977	161	167	(K) <u>YLVEIAR</u> (R)	1SMT
1217.5769	1217.5665	8.5367	223	228	(R) <u>CASIQK</u> (F)	2SMT
1233.5978	1233.6097	-9.6395	156	160	(K) <u>KFWGK</u> (Y)	2SMT
1308.5650	1308.5717	-5.0955	499	507	(K) <u>CCTESLVNR</u> (R)	1SMT
1567.8258	1567.8273	-0.9497	361	371	(R) <u>HPEYAVSVLLR</u> (L)	1SMT
1569.8294	1569.8218	4.8352	233	241	(R) <u>ALKAWSVAR</u> (L)	2SMT
1723.9240	1723.9284	-2.5523	360	371	(R) <u>RHPEYAVSVLLR</u> (L)	1SMT
1763.9128	1763.9121	0.4075	421	433	(K) <u>LGEYGFQNALIVR</u> (Y)	1SMT
1795.9618	1795.9594	1.3222	438	451	(K) <u>VPQVSTPTLVEVSR</u> (S)	1SMT
1851.8737	1851.8594	7.7344	347	359	(K) <u>DAFLGSFLYEYSR</u> (R)	1SMT
2208.1692	2208.1705	-0.5801	437	451	(R) <u>KVPQVSTPTLVEVSR</u> (S)	2SMT

The matched peptides cover 17% (109/607 AA's) of the protein

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Fig.13.



## Fig.14.

7/56 matches (12%). 35691.1 Da, pI=8.52. Acc. # swiss:P46406. RABIT. GLYCERALDEHYDE  
3-PHOSPHATE DEHYDROGENASE (EC 1.2.1.12) (GAPDH)..

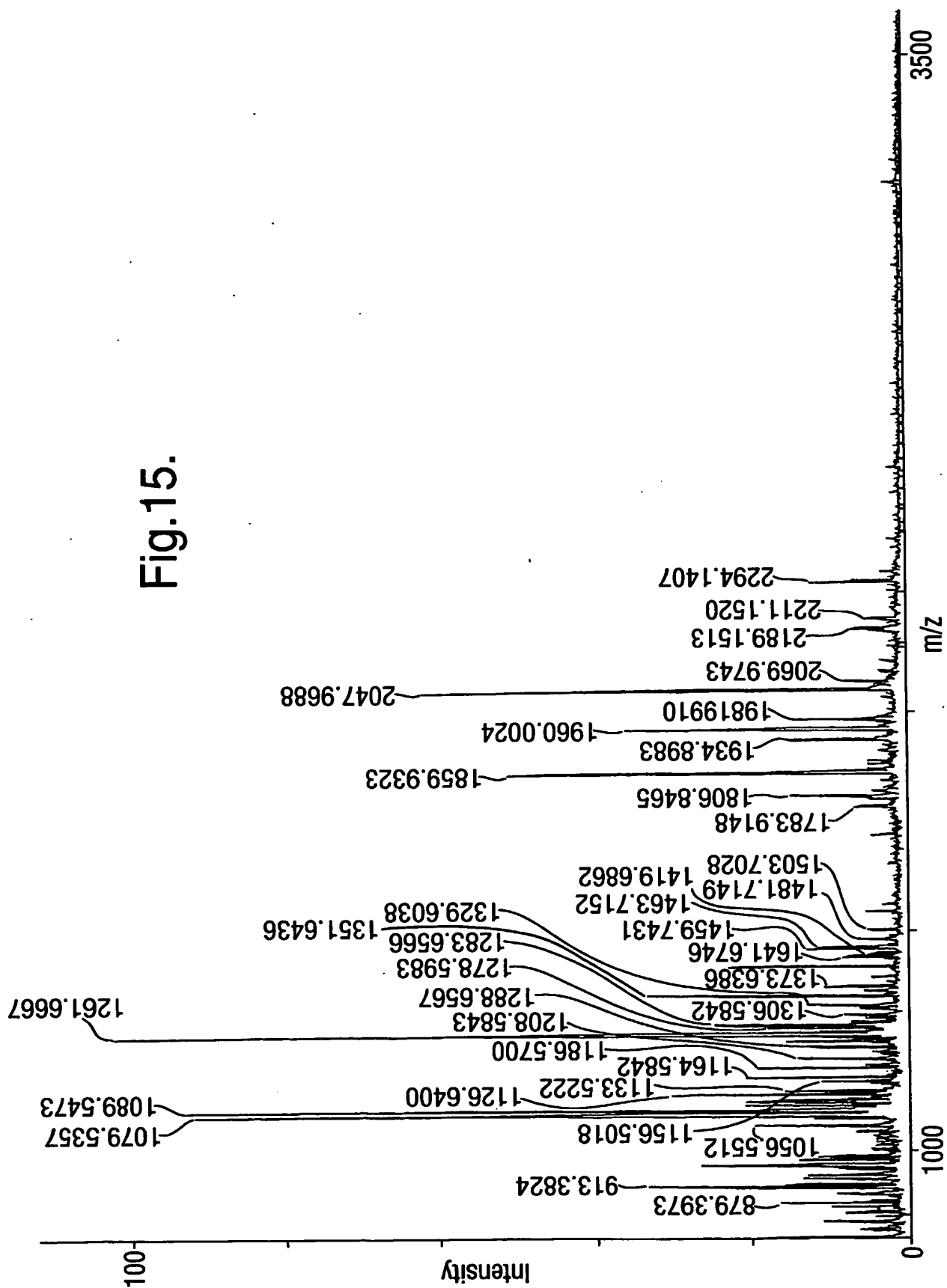
m/z	MH <sup>+</sup>	Δppm	start	end	Peptide Sequence	Modifications
submitted	matched					
977.5324	977.5420	-9.8265	70	77	(K)AITIFQER (D)	
1499.7853	1499.7892	-2.5929	232	245	(R)VPTPNVSVDLTCR (L)	
1615.8755	1615.8808	-3.2682	70	83	(K)AITIFQERDPANIK (W)	
1763.8175	1763.8029	8.2636	307	320	(K)LSWYDNEFGYSNR (V)	
2618.3883	2618.3765	4.5189	160	183	(K)VIHDFGIVEGLMTTVHAITATQK (T)	
3317.5310	3317.5651	-10.2886	25	52	(K)VDWAINDPFIDLHYMVYMFQYDSTHGK (F)	
3339.6063	3339.6432	-11.0492	84	115	(K)WGDAGAEVWESTGVFTTMEKAGAHKGGAKR (V)	1Met-ox

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The matched peptides cover 37% (126/332 AA's) of the protein

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Fig.15.





## Fig.16.

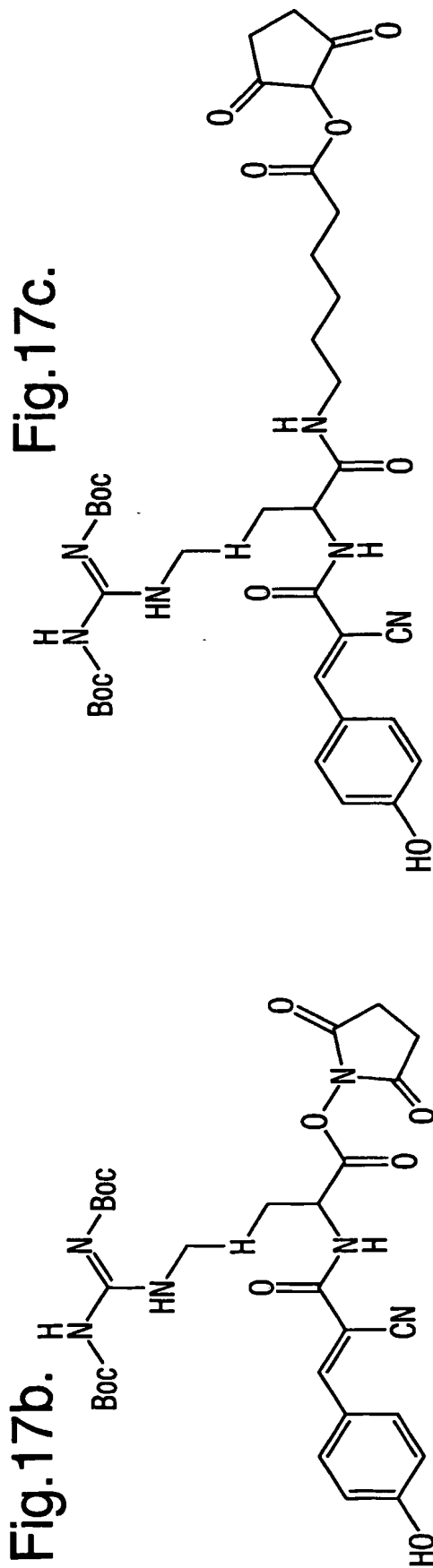
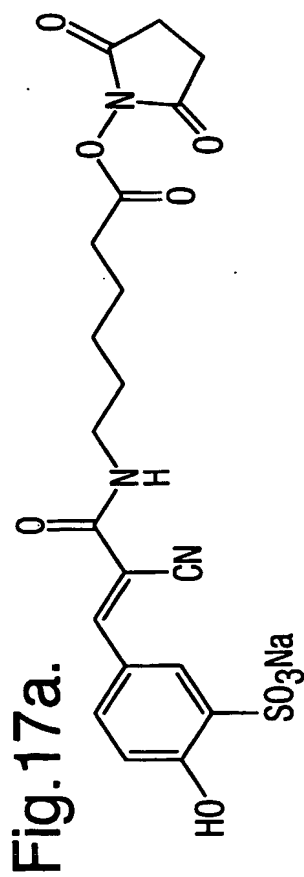
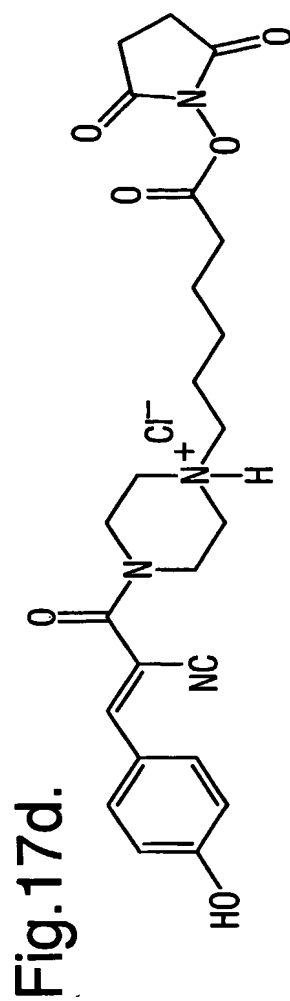
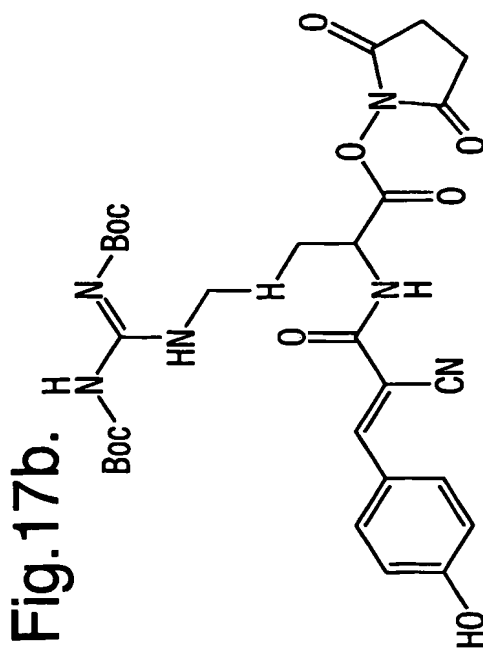
9/52 matches (17%). 35691.1 Da, pl=8.52. Acc. # swiss:P46406. RABIT. GLYCERALDEHYDE  
3-PHOSPHATE DEHYDROGENASE (EC 1.2.1.12) (GAPDH)..

m/z submitted	MH <sup>+</sup> matched	Δppm	start	end	Peptide Sequence	Modifications
1056.5512	1056.5267	23.1986	111	115	(K)GGAKR(V)	2SMT
1079.5357	1079.5348	0.8227	225	231	(K)LTGMAFR(V)	1SMT
1089.5473	1089.5482	-0.7836	3	10	(K)YGVNGFGR(I)	1SMT
1164.5842	1164.5842	-0.0042	105	110	(K)AGAHLK(G)	2SMT
1261.6667	1261.6581	6.8178	70	77	(K)AITIFQER(D)	1SMT
1783.9148	1783.8808	19.0702	184	194	(K)TVDGPSGKLWR(D)	2SMT
1783.9148	1783.9053	5.3359	232	245	(R)VPTPNVSWDLICR(L)	1SMT
1960.0024	1959.9897	6.4986	252	260	(K)YDDIKKWK(Q)	3SMT
1981.9910	1982.0096	-9.3660	195	212	(R)DGRGAAQNIIPASTGAAK(A)	1SMT
2047.9688	2047.9190	24.3090	307	320	(K)LSWYDNEFGYSNR(V)	1SMT

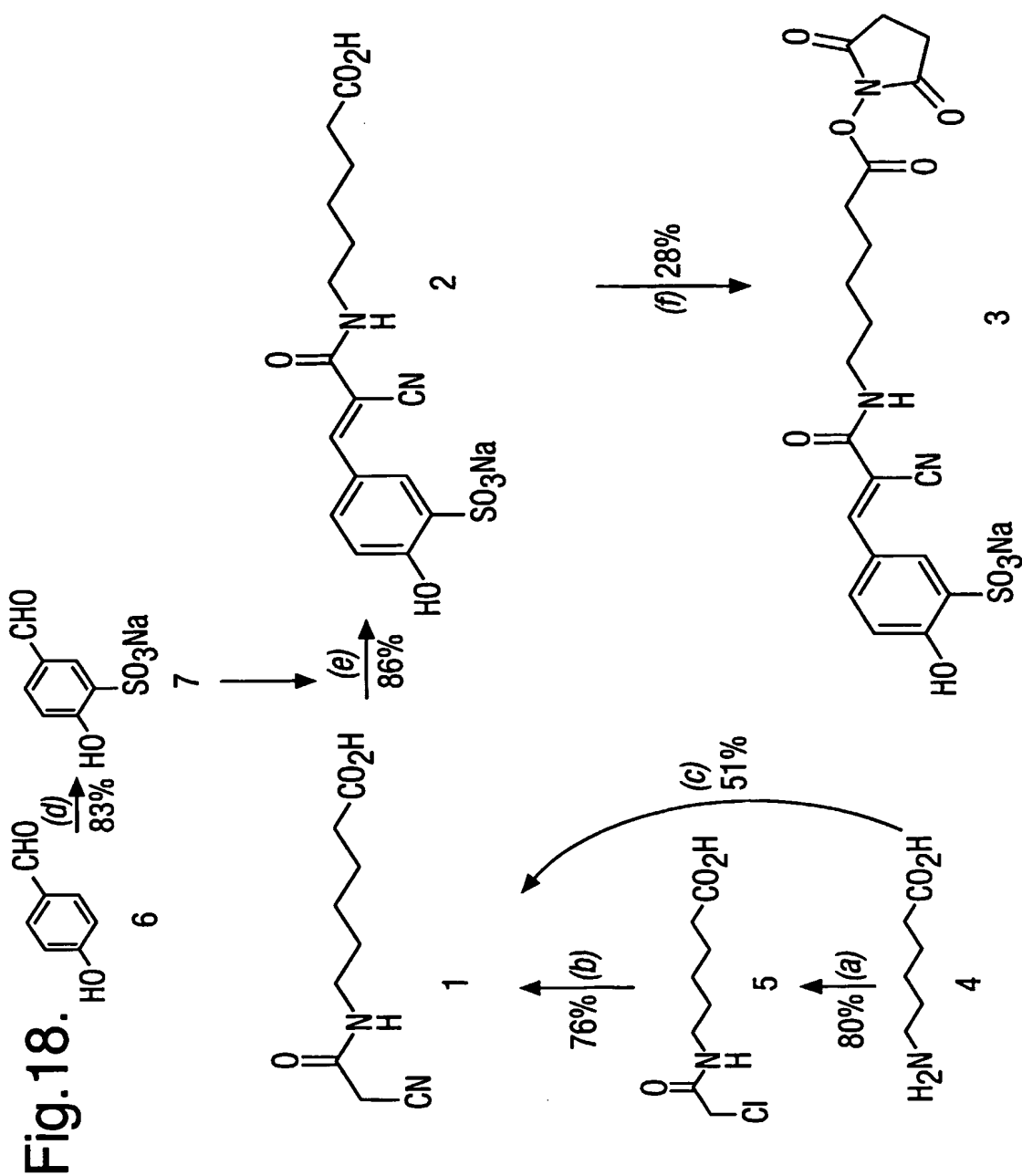
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The matched peptides cover 30% (100/332 AA's) of the protein

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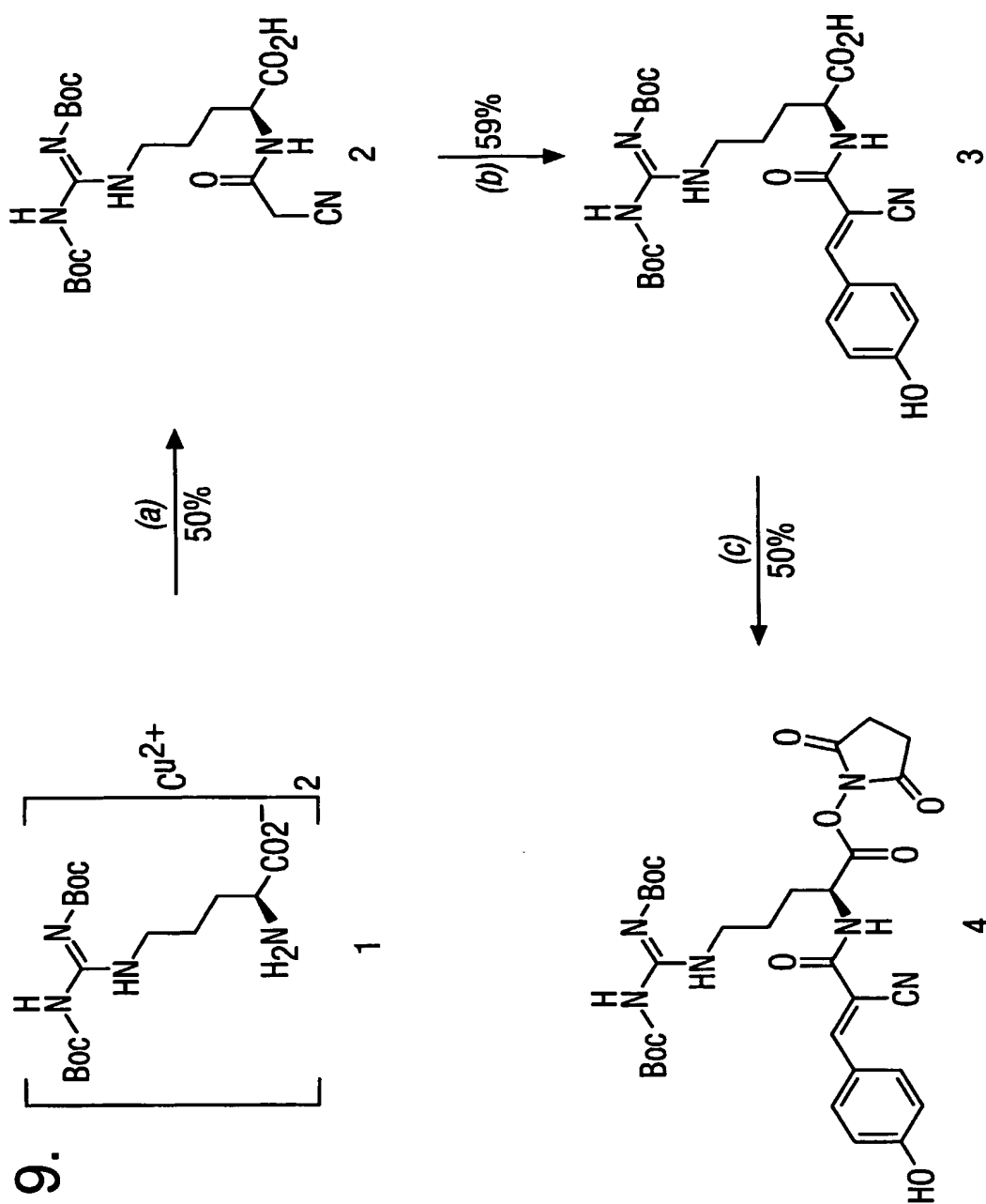
**Fig.17c.**

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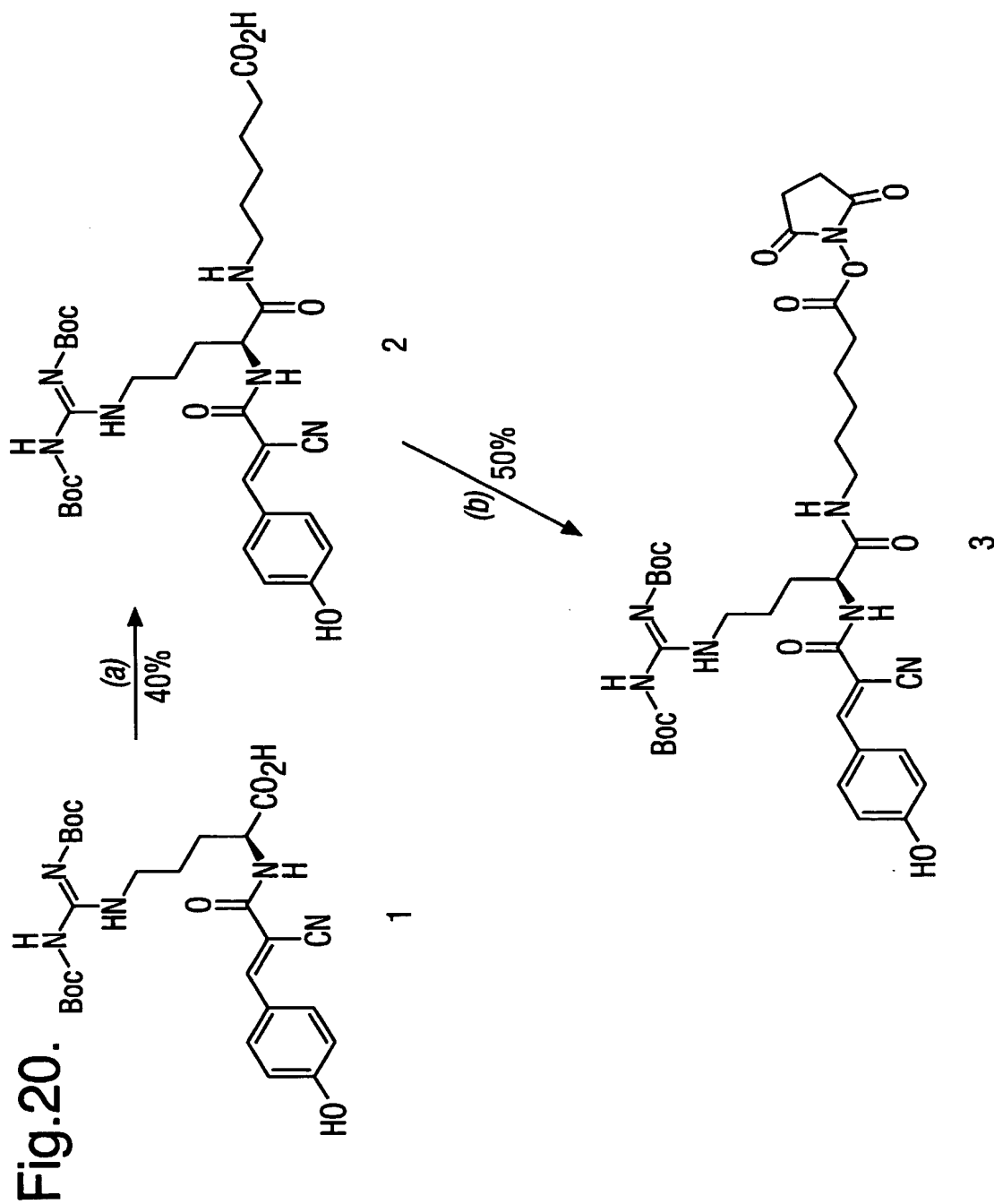
(a) Cl-CH<sub>2</sub>-COCl, TEA, CH<sub>2</sub>Cl<sub>2</sub>; (b) KCN, K<sub>2</sub>CO<sub>3</sub>, H<sub>2</sub>O; (c) NC-CH<sub>2</sub>-CO<sub>2</sub>Su, NaHCO<sub>3</sub>, THF/H<sub>2</sub>O;  
 (d) conc. H<sub>2</sub>SO<sub>4</sub>; (e) 7, Pyridine, [Piperidine]-HO-Su, DCC, Acetonitrile

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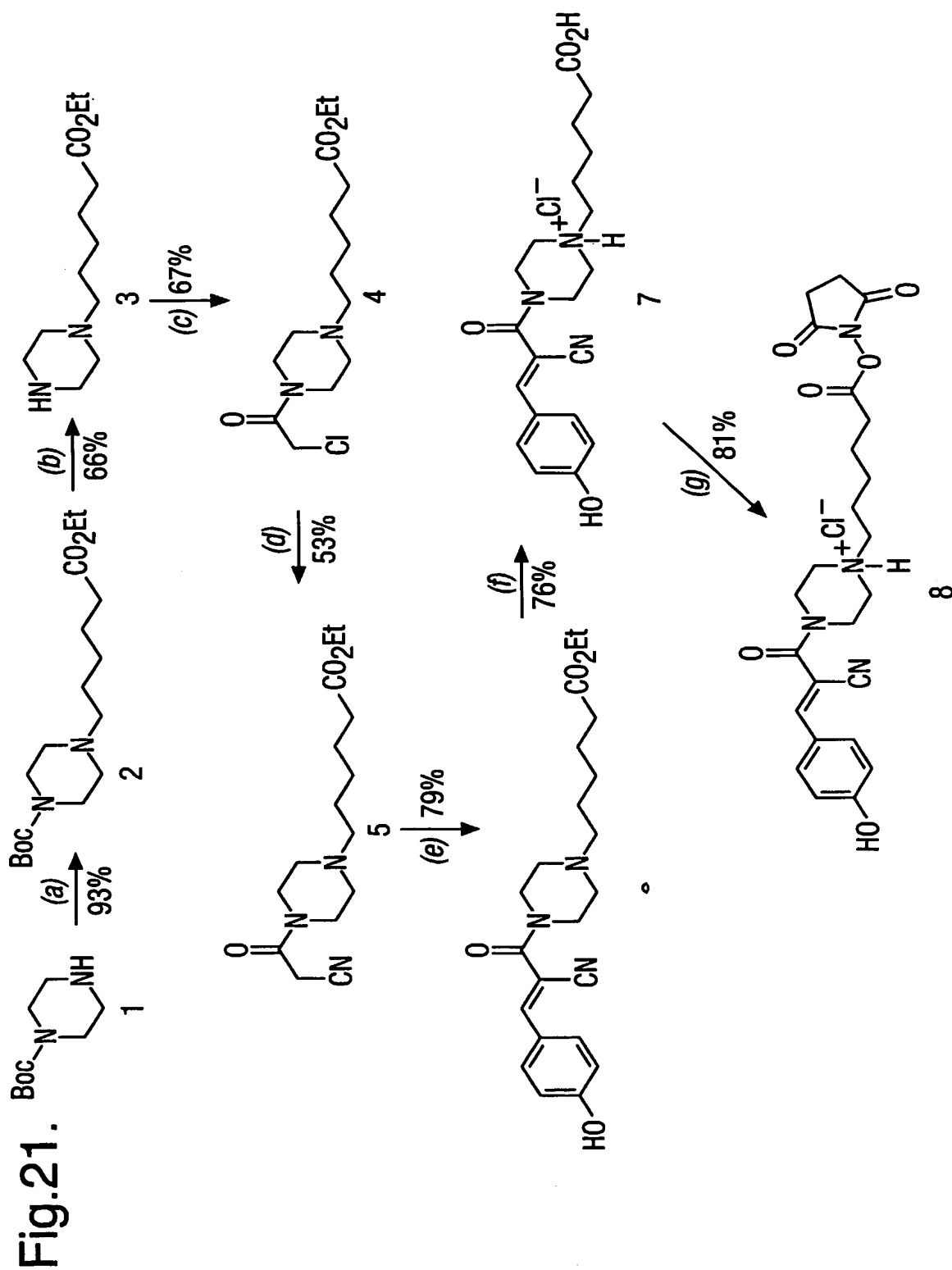
(a) EDTA-4Na·H<sub>2</sub>O, NaHCO<sub>3</sub>, NC-CH<sub>2</sub>-CO<sub>2</sub>Su, H<sub>2</sub>O/Acetone;  
 (b) HO-Su, DCC, CH<sub>2</sub>Cl<sub>2</sub>  
 (c) HO-C<sub>6</sub>H<sub>4</sub>-CHO, Pyridine, [Piperidine]

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(a) HO-Su, DCC, CH<sub>2</sub>Cl<sub>2</sub>, 6-Amino-hexanoic acid; (b) HO-Su, DCC, CH<sub>2</sub>Cl<sub>2</sub>

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(a) 1-(CH<sub>2</sub>)<sub>5</sub>-CO<sub>2</sub>Et, (isopropyl)<sub>2</sub>EtN, THF; (b) 2N HCl; (c) Cl-CH<sub>2</sub>-COCl, TEA, CH<sub>2</sub>Cl<sub>2</sub>; (d) KCN, DMSO; (e) 4-HO-C<sub>6</sub>H<sub>4</sub>-CHO, Pyridine, [Piperidine]; (f) 2N-NaOH/CH<sub>3</sub>OH; 2N-HCl; (g) HO-Su, DCC, DMF